NUMBER 13 JUNE, 1927

THE AMERICAN ANATOMICAL MEMOIRS

NUMBERS 1 TO 7, INCLUSIVE, APPEARED AS MEMOIRS OF
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

EDITED BY

CHARLES R. STOCKARD
CORNELL UNIVERSITY MEDICAL SCHOOL

AND

HERBERT M. EVANS
UNIVERSITY OF CALIFORNIA, BERKELEY

ON THE PROBLEM OF LYMPH FLOW BETWEEN CAPILLARIES OF THE BLOOD-VASCULAR SYSTEM AND BLINDLY-ENDING CAPILLARIES OF THE LYMPHATICS

CHARLES F. W. McCLURE
LABORATORY OF COMPARATIVE ANATOMY
PRINCETON UNIVERSITY

PUBLISHED BY
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA, PA., U.S.A.

Price, $2.00

The Wistar Institute of Anatomy and Biology
36th Street and Woodland Avenue :: :: Philadelphia, Pa.

Journal of Morphology and Physiology

Managing Editor

C. E. McClung
University of Pennsylvania, Philadelphia, Pennsylvania

Associate Editors

H. B. Ward
F. Payne
R. W. Hegner
M. H. Jacobs
L. J. Cole
J. H. Gerould
W. C. Curtis
J. Percy Moore
A. F. Shull

Published Quarterly
About 600 pages per volume

Price $12.00 per volume, Domestic
$12.50 per volume, Foreign

The Journal of Comparative Neurology

Editorial Board

C. J. Herrick, *Chairman of the Board*
Department of Anatomy, University of Chicago, Chicago, Illinois

Henry H. Donaldson
John B. Johnston
Adolf Meyer
Oliver S. Strong

Geo. E. Coghill, *Managing Editor*
The Wistar Institute, Philadelphia, Pennsylvania

Published Bi-monthly
About 500 pages per volume

Price $7.50 per volume, Domestic
$8.00 per volume, Foreign

The American Journal of Anatomy

Managing Editor

Charles R. Stockard
Cornell University Medical School, 28th St. and First Ave., New York

Associate Editors

Clarence M. Jackson
Harold D. Senior
George L. Streeter

Published Bi-monthly
About 500 pages per volume

Price $7.50 per volume, Domestic
$8.00 per volume, Foreign

THE WISTAR INSTITUTE PRESS

THE AMERICAN ANATOMICAL MEMOIRS

ON THE PROBLEM OF LYMPH FLOW BETWEEN CAPILLARIES OF THE BLOOD-VASCULAR SYSTEM AND BLINDLY-ENDING CAPILLARIES OF THE LYMPHATICS

CHARLES F. W. McCLURE

LABORATORY OF COMPARATIVE ANATOMY, PRINCETON UNIVERSITY

1927

PUBLISHED BY
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA

MEMOIR 13

TO MY WIFE

MARINE BIOLOGICAL LABORATORY
WOODS HOLE
LIBRARY
MASS.

CONTENTS

I. Introduction 7

II. The behavior of gloves of frog's skin, with the outside of the skin turned outward, when filled with Ringer's solution approximately isotonic with frog's blood, and suspended in the same solution 10

III. The behavior of healthy living frogs when transferred from water at one temperature to water at another 15

IV. The behavior of gloves of frog's skin, with the inside of the skin turned outward, when filled with Ringer's solution approximately isotonic with frog's blood and suspended in the same solution 16

V. The behavior of the gloves of frog's skin, with the outside of the skin turned outward, when filled with a hypotonic Ringer's solution and suspended in a hypertonic Ringer's solution approximately isotonic with frog's blood......... 27

  1. *The behavior of gloves of frog's skin filled with a Ringer's solution containing 6.5 grams of NaCl to 1000 cc. of H_2O, when suspended in a Ringer's solution containing 7 grams of NaCl to 1000 cc. of H_2O*.......................... 28

  2. *The behavior of gloves of frog's skin filled with a Ringer's solution containing 6.25 grams of NaCl to 1000 cc. of H_2O, when suspended in a Ringer's solution containing 7 grams of NaCl to 1000 cc. of H_2O*.......................... 30

  3. *The behavior of gloves of frog's skin filled with a Ringer's solution containing 6 grams of NaCl to 1000 cc. of H_2O, when suspended in a Ringer's solution containing 7 grams of NaCl to 1000 cc. of H_2O*.......................... 32

  Controls of above experiments: The behavior of gloves of frog's skin filled with a Ringer's solution containing 7 grams of NaCl to 1000 cc. of H_2O, when suspended in the same solution

VI. On the transport of water through the skin in the living frog with ureters ligated, when suspended in a hypertonic saline solution 34

VII. On the nature of the force which may drive water through the skin in a direction counter to that caused by osmotic pressure 38

VIII. On a possible explanation of the continuous flow of lymph between the capillaries of the blood-vascular system and those of the blindly-ending lymphatics.................... 50

BIOLOGICA
OODS
LIBRA
MASS

ON THE PROBLEM OF LYMPH FLOW BETWEEN CAPILLARIES OF THE BLOOD-VASCULAR SYSTEM AND BLINDLY-ENDING CAPILLARIES OF THE LYMPHATICS

I. INTRODUCTION

During the last twenty years, the writer has been specially interested in the morphology and the physiology of the lymphatic system. In the course of his investigations several problems have presented themselves. Among these is an inquiry into the causes of oedema, concerning which two papers have already been published.[1] Another problem concerned a possible provisional arrangement of the embryonic lymphatic system, by which could be explained the manner in which a centripetal lymph flow toward the venous circulation from the interstitial spaces could be controlled and regulated until the lymph could reach the venous circulation.

It is now well known that the anlagen of the lymphatic system do not make their appearance in the embryo until after the blood-vascular channels have been established. As soon, however, as the latter begin to function, lymph necessarily begins to collect in the interstitial spaces of the embryo, and accumulates there until it is subsequently collected by the newly formed set of blindly ending vessels, the lymphatics, by which it is conveyed to the venous circulation.

One of the salient facts observed in the development of the vascular system is that the main channels, both haemal and lymphatic, develop *in loco* from mesenchyme and are formed through the concrescence of independent and discontinuous anlagen. See McClure, 1921, for an historical account of the investigations bearing on this subject.[2]

---

[1] McClure, C. F. W. 1919 On the experimental production of oedema in larval and adult anura. Jour. Gen. Physiol., vol. I.

———— 1925 An experimental analysis of oedema in the frog, with especial reference to the oedema in red-leg disease. Amer. Anat. Mem., no. 12.

A study of living trout embryos made by the writer[3] has demonstrated that certain of these independent anlagen of the lymphatic system, the subocular lymph sacs, receive through their closed endothelial-lined walls lymph which flows from the interstitial spaces in a centripetal direction, and that the lymph received in these sacs is temporarily retained by them until the sacs secondarily join the lateral pharyngeal lymphatic (jugular), by which it is then transported to the venous circulation. Subocular lymph sacs were observed also in the embryos of Amia and Lepidosteus. In the embryos of ganoids, however, these sacs communicated directly with the veins at a very early stage of development, so that the lymph received in them was not retained, but was directly transported to the venous circulation. What we find to be the case in both trout and ganoids is that lymph from the tissue spaces flows in a centripetal direction through the endothelial-lined walls of blindly ending lymphatics, and that in the case of the trout this lymph is temporarily retained by closed and detached independent anlagen of the lymphatic system, until the conditions are such that the centripetal flow can be continued to the venous circulation. It can therefore actually be demonstrated in the living trout embryos not only that certain lymphatics end blindly, but also that certain of the independent anlagen of the lymphatic system serve as reservoirs for the reception and temporary retention of lymph received from the interstitial spaces prior to the establishment of continuous lymphatic channels, by which it is subsequently conveyed to the venous circulation. To what extent independent endothelial-lined and closed lymph sacs of the embryo, other than the subocular sacs of the trout, function in a similar manner during ontogeny, is a question still to be determined. In any event, we can now demonstrate in the living trout embryo that lymph in the interstitial spaces received from the blood capillaries collects in certain closed lymph sacs which are at first independent of the veins and of other lymphatics.

---

[2] McClure, C. F. W. 1921 The endothelial problem. President's address: read before the American Association of Anatomists in Philadelphia. Anat. Rec., vol. 22.

[3] McClure, C. F. W. 1915 The development of the lymphatic system in fishes with especial reference to its development in the trout. Amer. Anat. Mem., no. 4.

——— 1915 On the provisional arrangement of the embryonic lymphatic system. An arrangement by means of which a centripetal lymph flow toward the venous circulation is controlled in an orderly and uniform manner, from the time lymph begins to collect in the intercellular spaces until it is forwarded to the venous circulation. Anat. Rec., vol. 9.

It was largely through these researches on the development of the lymphatic system that the writer was led to study the problem of the flow of lymph between the blood capillaries and the lymphatics; and his investigation is set forth in the following pages. The study concerns chiefly the capacity of frog skin (whether it be the skin of the living animal or skin detached from the body) to transport water through itself under various experimental conditions. Many observations pertinent to the present study have been made by previous writers; the main feature of the present work is its analysis of an extended series of observations, which indicates a possible explanation of the continuous flow of fluid between the blood capillaries and the blindly ending capillaries of the lymphatics.

We know that water is chiefly supplied to the body of the living frog by osmosis through the integument into the subcutaneous lymph sinuses. Reid,[4] Overton,[5] and Maxwell[6] have observed that when a skin glove, with the outside of the skin turned outward, is filled with a Ringer's solution and suspended in the same solution, water may, in spite of the equilibrium of osmotic pressure, be transported through the skin into the glove.

---

[4] Reid, E. W. 1890 Osmosis experiments with living and dead membranes. Jour. Physiol., vol. XI.

[5] Overton, E. 1904 Neununddreissig Thesen über die Wasserökonomie der Amphibien und die osmotischen Eigenschaften der Amphibienhaut. Verhl. d. Phys.-med. Ges. zu Würzburg, N.F., Bd. 36.

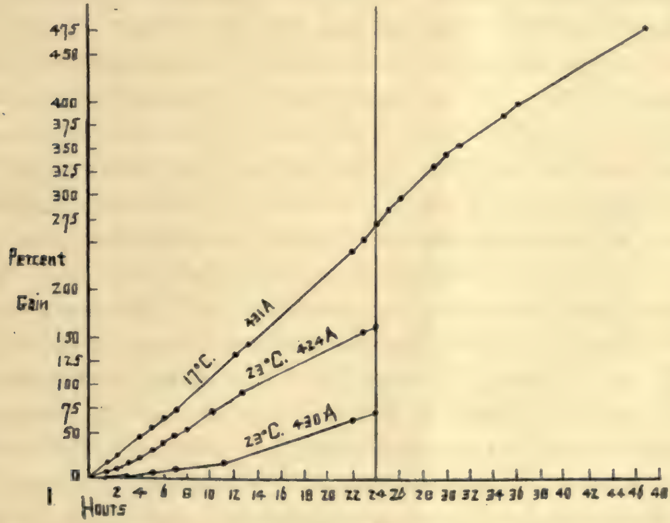
[6] Maxwell, S. S. 1913 On the absorption of water by the skin of the frog. Amer. Jour. Physiol., vol. 32.

It has often been stated that the skin of frogs of the same as well as of different species may vary greatly in its general character, especially as regards permeability. Since we now recognize the fact that water is transported through the skin into the glove when the latter, with the outside of the skin turned outward, is filled with a Ringer's solution and is suspended in the same solution, it seems probable that a study of frog skins, as regards their relative permeability, might throw a new light on still other phenomena associated with the transport of fluid through the skin.

The technique employed in the preparation of the skin gloves used in this investigation was essentially the same as that employed by other investigators. In most cases the skin of the hind legs was used in the form of a glove, a ligature being applied to the opening left where the skin had been cut off at the ankle. The upper end of the glove was tied to a glass tube, which was then closed by a paraffined cork to prevent evaporation of fluid placed in the glove when the latter was suspended in solutions. This method of preparation allows the experimenter to change the fluid in the glove, and so to make use of the same glove for different experiments. At stated intervals the gloves were removed from the solutions and were weighed. Always the outside of the skin was dried rapidly with filter-paper before the weighing was done. The experimental error involved in weighing skin gloves obtained from the hind legs of small frogs amounted to about 5 milligrams.

II. THE BEHAVIOR OF GLOVES OF FROG'S SKIN, WITH THE *OUTSIDE* OF THE SKIN TURNED *OUTWARD*, WHEN FILLED WITH RINGER'S SOLUTION APPROXIMATELY ISOTONIC WITH FROG'S BLOOD, AND SUSPENDED IN THE SAME SOLUTION

The writer has made a large number of these experiments. They were made in every month of the year; the skins used were taken from living frogs which had been recently captured, and also from frogs which remained in captivity without food for several months prior to the experiments. The normal average behavior of the skin gloves has been graphically represented in figures 1, 2, 3, and 4. *In these experiments, and in all others of a similar sort, the percentage of the gain or the loss in weight of the skin glove, unless otherwise stated, has been estimated upon the original weight of the empty skin glove at the time of its removal from the body—that is, before fluid was placed in the glove and before the glove was suspended in the solution. For example, in experiment 431 A (fig. 1) a gain in weight of the skin glove amounting to about 475 per cent indicates that during a period of forty-eight hours the weight of the fluid transported through the skin into the glove amounted to 475 per cent of the weight of the empty skin glove before it was filled with Ringer's solution and before it was suspended in the solution.*



1

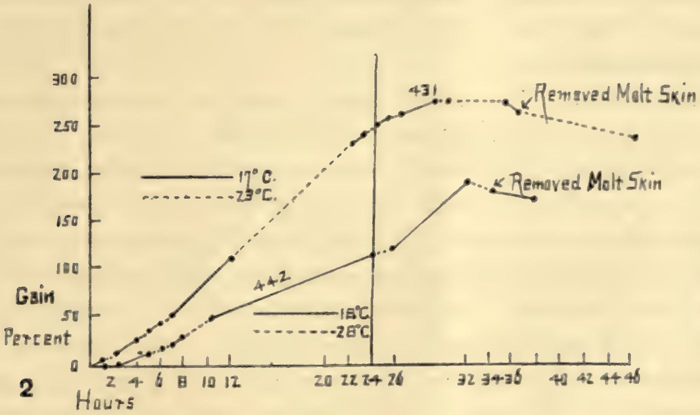
Figure 1 represents the range of behavior observed in the case of three skin gloves removed from the hind legs of three different healthy frogs (R. pipiens) which had been supplied by a dealer about one month prior to the experiments. Each skin glove, with the outside of the skin turned outward, was filled with Ringer's solution approximately isotonic with frog's blood, and was suspended at a *constant* temperature in

MARINE BIOLOGICA
WOODS HO
LIBRA
MASS

the same solution. It will be observed that, during a period of twenty-four hours' suspension in the solution, these three gloves showed marked differences in the rate at which water was transported through the skin from without inward. In one case the gain in weight of the glove amounted to 272.2 per cent; in the two remaining gloves it amounted to 160.7 and 68 per cent, respectively. In cases where the rate of passage of water through the skin was relatively rapid, it was found that the glove continued to gain in weight for a period of forty-eight hours or more, although the gain was less on the second than on the first day of suspension. These three experiments showed that, among different frogs of the same species, during the same season of the year, marked differences exist in the rate at which water is transported through the skin into the glove under the conditions above mentioned.

When skin gloves, with the outside of the skin turned outward, are filled with Ringer's solution approximately isotonic with frog's blood and are alternately suspended in the same solution at *different* temperatures, their behavior may be the same during the first day's suspension as if the temperature of the Ringer's solution had been constant (experiments 431 and 442, fig. 2). Also, the rate at which water is transported through the skin into the glove is not invariably influenced by temperature, in the sense that the gain in weight of the glove is greater at a high than at a lower temperature. As a matter of fact, instances were frequently met with in which the reverse conditions were observed.

During the second day of their suspension in Ringer's solution at different temperatures, the skin gloves usually showed signs of molting, and the rate at which water was then transported through the skin into the glove became greatly diminished. After the loss of the superficial layer of skin (molt skin), a complete reversal was brought about in the direction in which the transport of water through the skin was accomplished. In such cases water was transported through the skin *outward* instead of *inward*. The behavior of skin gloves during the second day of their suspension in isotonic Ringer's solution and after molting is shown in figure 2. In experiments 431 and 442 (fig. 2) the molt skin was removed from the glove at the time indicated in the figure, after which the gloves began to lose in weight continuously. A comparison of experiment 431 A (fig. 1) with experiment 442 (fig. 2) illustrates the behavior of two skin gloves taken from the same frog when filled with isotonic Ringer's solution and suspended in the same solution at a constant (431 A) and at different (442) temperatures. The time of molting naturally varies greatly in different cases, and is largely influenced by the temperature of the solution in which the glove is suspended. At the time of their removal from the body, skins in which the rate of transport of water is relatively slight show signs of molting earlier than those in which the rate is higher.



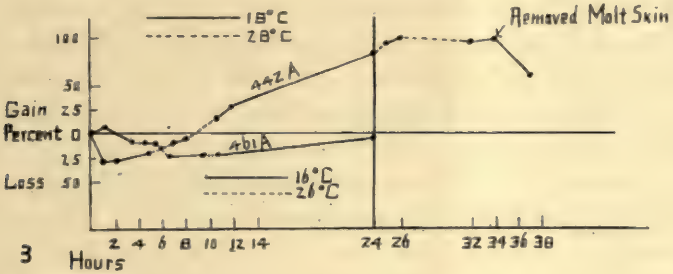
2

In most cases skin gloves, with the outside of the skin turned outward, filled with isotonic Ringer's solution, immediately begin to gain in weight and continue to do so for a considerable time, either at a constant or at varying temperatures, when suspended in the same solution. Exceptions to this behavior are, however, found. In some instances a loss in weight, instead of a gain, immediately succeeds the suspension of the glove in Ringer—though such temporary loss is almost invariably succeeded by a gain. This form of behavior is illustrated by experiment 442 A (fig. 3), in which the initial loss in weight of the glove amounted to 25 per cent during the first hour of its suspension in Ringer; then a gain in weight followed up to the twenty-sixth hour, when the weight remained stationary until the molt skin was removed from the glove. The behavior of the glove in experiment 461 A (fig. 3) differs slightly from that observed in the last-mentioned experiment. In experiment 461 A a very slight gain in the weight of the glove, during the first hour of its suspension in Ringer, was succeeded by a loss which continued for five hours, after which there was a slight gain. The two frogs from which these skins were taken were very thin and weak, and, in experiment 461 A, the frog used was quite emaciated.

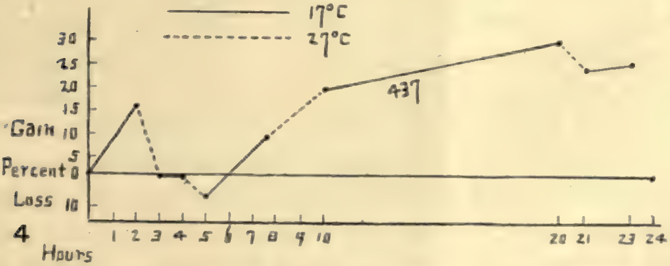
In figure 4 is graphically shown another experiment (437) made on a skin glove removed from the hind leg of a frog (R. pipiens) which was thin and which had molted just prior to the removal of the skin from the body. This experiment is interesting as showing the influence of temperature on the weight of a glove with the outside of the skin turned outward, filled with Ringer's solution approximately isotonic with frog's blood and alternately suspended in the same solution at different temperatures. During the first five hours of its suspension in Ringer at 17°C., the glove either gained or remained stationary in weight; when transferred to the same solution at 27°C., however, the glove lost. Between the fifth and twentieth hours, the gain in weight of the glove was continuous, irrespective of differences in temperature; after this, however, in relation to temperature changes, the weight showed fluctuations similar to those of the first five hours. While this type of behavior does not appear to be especially common, a sufficiently large number of cases has been observed to justify placing it among the typical forms which one occasionally encounters. Perhaps the most important and significant feature of this last-mentioned behavior is that exactly similar fluctuations in body weight can be produced in the living frog when the latter is transferred from water at one temperature to water at another.

III. THE BEHAVIOR OF HEALTHY LIVING FROGS WHEN TRANSFERRED FROM WATER AT ONE TEMPERATURE TO WATER AT ANOTHER

In one hundred experiments made by the writer, in which normal healthy frogs were transferred from water at a low to water at a higher temperature, seventy-three lost and twenty-seven gained weight immediately after the transfer. The average initial maximum loss in weight amounted to 5.9 per cent, and the average maximum gain to 3.22 per cent.



3



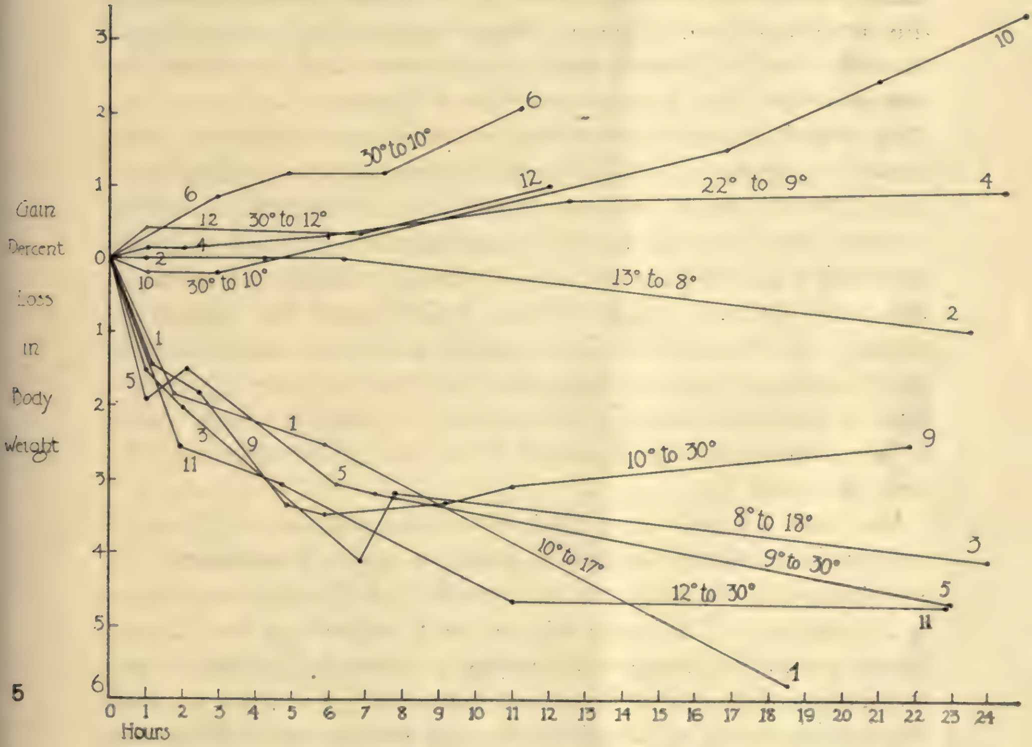
4

Also, in sixty-six experiments, in which normal healthy frogs were transferred from water at a high to water at a lower temperature, fifty-six, or about 85 per cent, gained, and ten, or about 15 per cent, lost weight immediately after the transfer. The average initial maximum gain in weight amounted to 3.79 per cent and the average initial maximum loss to 2.44 per cent.

The fluctuations in body weight which resulted when a living frog (R. pipiens, no. 246) was alternately transferred from water at one temperature to water at another are graphically represented in figure 5. The numerals opposite each curve in figure 5 indicate the order in which the experiments were made. For example, the first transfer (curve *1*) was made from water at 10°C., in which the frog had previously remained for a considerable time, to water at 17°C. The second transfer (curve *2*) was made from water at 13°C. to water at 8°C., and so on. Between experiments 6 and 9 two desiccation recovery curves (McClure, '25, fig. 1), were made, based on the reactions of the same frog. These are not included in the figure. The odd numbers (curves *1, 3, 5, 9,* and *11* in fig. 5) represent the behavior of the frog when transferred from water at a *low* to water at a *higher* temperature. The even numbers (curves *2, 4, 6, 10,* and *12* in fig. 5) represent the behavior of the frog when transferred from water at a *high* to water at a *lower* temperature. Had it been practical to do so, the behavior of this frog would have been graphically represented in one curve. In the present case the zero point on the ordinate marks the beginning of each transfer. This series of experiments extended over a period between March 26th and April 26th, and was made on a frog obtained from a dealer in February. During the course of the last experiment (curve *12*) the frog contracted red-leg disease, and its gain in weight then became continuous (McClure, l.c., '25). This last experiment (curve *12*) extended over a period of 170 hours, at the end of which time the gain in weight of the frog amounted to 32.8 per cent—a gain not graphically represented in the figure. Since a continuous increase in body weight in the frog is coincident with the appearance of oedema, the writer deemed it worth while to make an analysis of these fluctuations observed both in the skin and in the living frog.

IV. THE BEHAVIOR OF GLOVES OF FROG'S SKIN, WITH THE *INSIDE* OF THE SKIN TURNED *OUTWARD,* WHEN FILLED WITH RINGER'S SOLUTION APPROXIMATELY ISOTONIC WITH FROG'S BLOOD AND SUSPENDED IN THE SAME SOLUTION

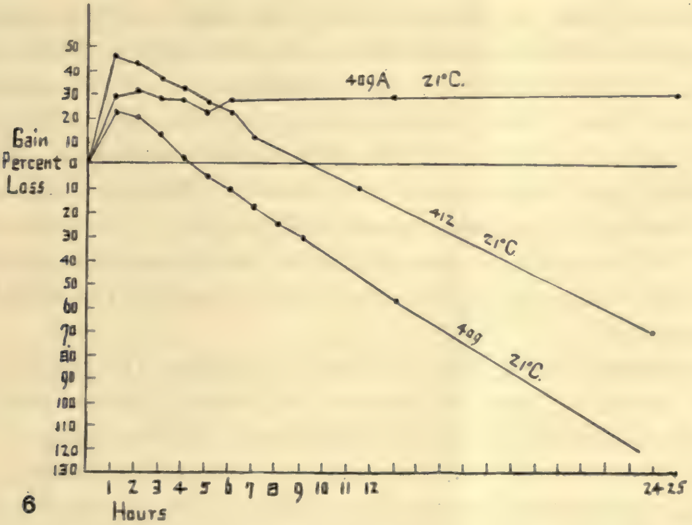
When skin gloves, with the *inside* of the skin turned *outward,* are filled with isotonic Ringer's solution and suspended in the same solution, there is, as numerous investigators have observed, a general tendency for water to pass from the glove through the skin outward, in a direction exactly opposite to that which occurs when the outside of the skin is turned outward. This transport of water outward through the skin is usually not at first evident, for the reason that the glove almost invariably gains weight during the first hour of its suspension in Ringer's solution. This gain in weight is not due, however, to the presence of water which has passed from without inward through the skin into the glove, but rather to the fact that the skin itself has absorbed and held water, in excess of that which is simultaneously transported from the glove through the skin outward. When the hydration capacity of the skin has been satisfied so that water is no longer absorbed by the skin itself, then the glove often begins to lose weight, due to a passage of water from the



glove through the skin outward. In other words, if the skin itself did not at first absorb and hold water, the glove would probably begin to lose weight immediately when suspended in Ringer's solution. It is therefore evident that at the start at least two separate factors acting in opposition to each other are involved in the behavior of skin gloves, with the *inside* of the skin turned *outward,* when filled with Ringer's solution isotonic with frog's blood and suspended in the same solution. One of these factors is the capacity of the skin itself to absorb and to hold water, and the other, the power of the skin to transport water through itself, when the skin is bathed on both surfaces by solutions having the same osmotic pressure. While both factors may act simultaneously, the action of one may mask or overbalance the other, so that, even though water is transported through the skin outward when the glove is first placed in Ringer's solution, the glove will not begin to lose weight until the hydration capacity of the skin has been satisfied and an equilibrium in this respect has been established—or, until the rate of absorption of water by the skin is exceeded by that at which water is simultaneously transported from the glove through the skin outward.

We have already seen that, when the outside of the skin is turned outward, the rate at which water is transported inward through the skin varies greatly in different cases (figs. 1, 2, 3, and 4). The same may be said concerning the rate at which water is transported outward through the skin when the inside of the skin is turned outward. We now pass to the consideration of a number of experiments which illustrate the behavior of skin gloves with the *inside* of the skin turned *outward,* when filled with Ringer's solution approximately isotonic with frog's blood and suspended in the same solution.

Experiments 409 and 412 (fig. 6) illustrate the behavior of skin gloves removed from the hind legs of two different frogs (R. pipiens) which had been kept in the laboratory during the winter prior to the experiments. The frogs were active, but their skin was pale and dull in color. In each case, the glove, with the *inside* of the skin turned *outward,* was filled with isotonic Ringer's solution and suspended in the same solution at room temperature. At the end of the first hour of suspension, the gain in weight amounted in one glove (exper. 409) to 23.8 and in the other (exper. 412) to 47.5 per cent. After the first hour, however, each glove began to lose weight, through the transport of water from the glove through the skin outward. At the end of twenty-four hours' suspension in Ringer, the loss in one case amounted to 119 (exper. 409) and in the other to 70.5 per cent (exper. 412). If we compare the behavior of these two gloves filled with Ringer's solution with that of an empty glove (exper. 409, A, fig. 6) taken from the hind leg of one of these same frogs (409), also with the *inside* of the skin turned *outward,* we observe that while the empty glove gained weight (31.6 per cent) during the first two hours of its suspension in Ringer's solution, after this time its weight remained practically stationary. In this instance the empty skin glove contained no fluid which could be transported from the glove through the skin outward and, since no fluid was transported through the



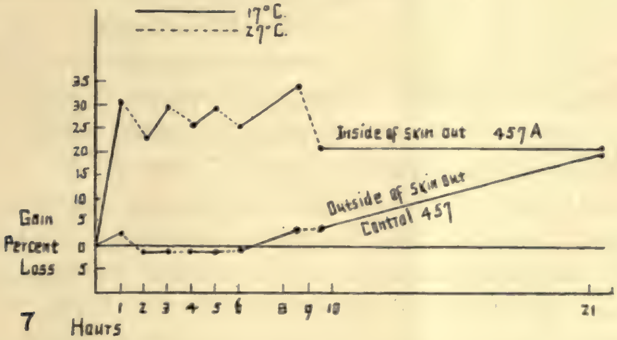
6

skin in the opposite direction, the gain in weight of the glove must have been due to the presence of fluid which had been absorbed by the skin and held by the latter. We have reason to suppose that if this same skin had been filled with Ringer's solution, its behavior would not have differed essentially from that of the glove which had been obtained from the same frog, but which had been filled with Ringer's solution (exper. 409).

When a living frog remains out of water, we know that as the result of loss of water from the body the osmotic pressure of its body fluid may be raised. The same may be said of skin gloves, which undoubtedly lose some water during the handling in preparation for the experiments. While osmosis may play some rôle in accounting for the fact that skin gloves, with the inside of the skin turned outward, almost invariably gain weight when first placed in Ringer's solution, the following experiments seem still further to bear out the view that this gain is due chiefly to the absorption of fluid by the skin until its tissue colloids have reached their hydration capacity.

Experiment 457 A (fig. 7) is one of interest for the reason that it shows a form of behavior indicating that a gain or a loss in weight of the glove is influenced by temperature, but cannot be explained on the grounds of osmotic pressure.

In this experiment a skin glove, with the *inside* of the skin turned *outward,* which had been removed from the right hind leg of a frog (R. pipiens) kept in the laboratory for several months prior to the experiment, was filled with Ringer's solution approximately isotonic with frog's blood and was alternately suspended in the same solution at 17°C. and at 27°C. During the first hour of its suspension at 17°C. the glove gained about 30 per cent, because the skin itself had a marked capacity to absorb and hold water. During a period of eight hours' suspension following this initial gain, the glove uniformly lost weight when transferred to Ringer's solution at the high temperature (27°C.) and gained weight when transferred from this solution to one at the low temperature (17°C.). As previously stated, during the first hour the gain in weight of the glove may have resulted partly from differences in osmotic pressure of the fluid in the skin and that of the solution in which the glove was suspended; but it would be difficult to explain its succeeding behavior as an osmotic reaction. The loss in weight of the glove during the second hour indicates that the hydration capacity of the skin had previously been satisfied; and the fact that a loss in weight regularly occurred when the glove was suspended in Ringer at 27°C., and a gain when it was suspended in Ringer at 17°C., indicates that the gain and the loss in weight were due not to osmotic pressure, but rather to the gain and the loss of water by tissue colloids.



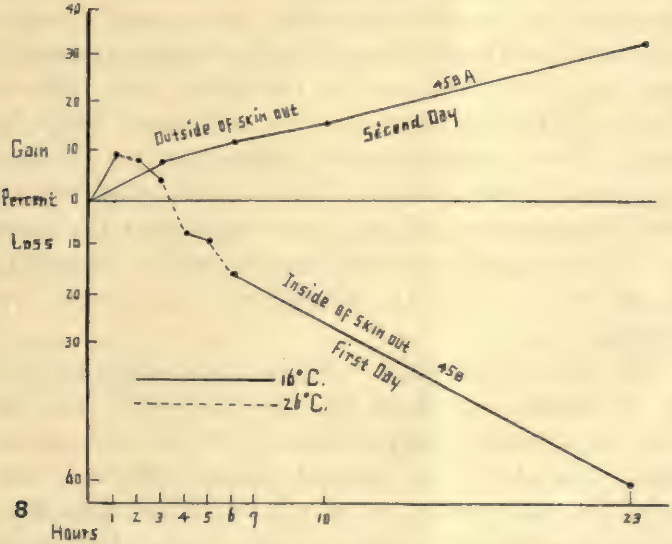
7

The control of this experiment (457, fig. 7), was the skin glove from the left hind leg of the same frog, with the *outside* of the skin turned *outward;* this skin was filled with Ringer's solution approximately isotonic with frog's blood, and was then suspended at different temperatures in the same solution. The behavior shows that during the first six hours there was not a continuous transport of water through the skin and that even at the end of twenty-four hours' suspension the gain in weight of the glove was insignificant. Since in the control the normal direction in which water is transported through the integument is from without inward, we may infer that in the control the loss in weight observed during the second hour is due to a loss of water by the tissue colloids rather than to osmotic pressure. The same is observed in other cases (fig. 4) where the outside of the skin is turned outward.

Although the behavior just mentioned (exper. 457 A, fig. 7) has frequently been observed, it is by no means uniformly met with. As in the case of skin gloves with the outside of the skin turned outward (fig. 2), cases are found in which fluid is transported through the skin in a continuous manner when gloves filled with Ringer's solution approximately isotonic with frog's blood, with the *inside* of the skin turned *outward,* are alternately suspended in the same solution at different temperatures. This behavior is shown in the following experiment.

Experiment 458 (fig. 8) was made on a skin glove removed from the right hind leg of a frog (R. pipiens). The glove, with the *inside* of the glove turned *outward,* was filled with 6 cc. of Ringer's solution isotonic with frog's blood, and then was alternately suspended for definite periods of time in this same solution at temperatures of 16°C. and 26°C. After the usual initial gain in weight obtained during the first hour's suspension, the glove lost weight continuously at both low and high temperatures, and in this respect its behavior differed from that observed in experiment 457 A (fig. 7). The loss in weight of the glove was continuous; but it will be noted that during certain hourly periods the loss was greater at 26°C. than at 16°C. This may be accounted for as follows: at 16°C. and at 26°C. water was transported from the glove through the skin outward. At 16°C., however, the skin itself absorbed, and at 26°C. it lost water. In this way it may be possible to explain the greater loss at the higher temperature.

After twenty-three hours' suspension in Ringer with the inside of the skin turned outward, the glove was removed from the glass cylinder, and both surfaces of the skin were carefully dried between sheets of filter-paper. The same glove was then again fastened to a glass tube with the *outside* of the skin turned *outward,* instead of *inward,* was filled with 6 cc. of Ringer's solution isotonic with frog's blood, and was suspended in the same solution at 16°C. The behavior of the glove (458 A), with the outside of the skin turned outward, was typical of that observed in most experiments of this character. It gained weight continuously from the time it was first suspended in Ringer's solution (fig. 8, second day). A comparison of the behavior observed on the first and on the second days clearly still further emphasizes the fact that water is more readily absorbed by the skin through its inner surface than through its outer, and that the direction in which water is transported through the skin in opposition to osmotic pressure depends upon which surface of the glove is turned inward or outward.



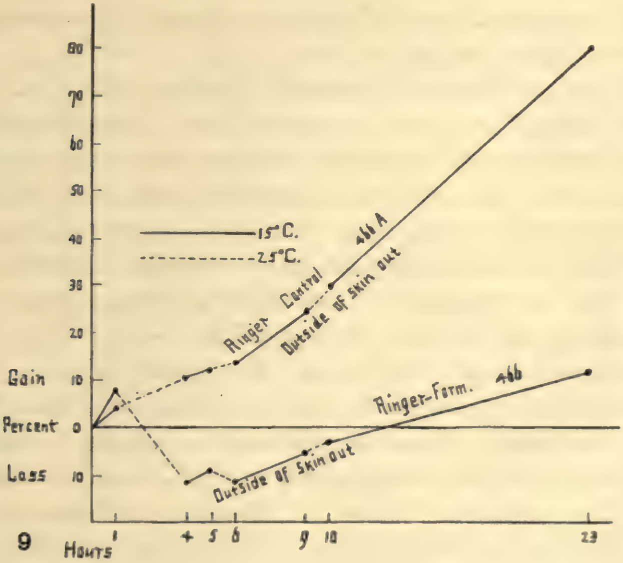
8

As all of the experiments mentioned in the preceding pages were made on skins removed from living frogs, we may infer that the behavior observed, at least during the first few hours of the experiment, represents the average condition one would find in the power of the living skin both to absorb water and to transport water through itself in opposition to osmotic pressure. It has been observed in the majority of instances that water is transported through the skin in a definite direction and in a continuous flow when skin gloves filled with Ringer's solution approximately isotonic with frog's blood are suspended in the same solution either at a constant temperature or at different temperatures. Also, when fluctuations in the weight of the glove occur, in the sense that the latter loses or gains weight in relation to differences in temperature, we have seen that the fluctuations appear to occur more readily in skins whose power to transport water through them in opposition to osmotic pressure either is lost or has become greatly diminished. If, therefore, by means of experiment we can eliminate the continuous transport of water through the skin in opposition to osmotic pressure, and if a gain or a loss of water by the glove then occurs, this must be due to the absorption or to the loss of water by the skin itself. When formaldehyde is added both to the Ringer's solution placed in the glove and also to the solution in which the glove is suspended, it has been found that the power of the skin to transport water through itself in opposition to osmotic pressure either is completely lost or is greatly diminished.

Two skin gloves removed from the right and the left hind legs of the same individual (R. pipiens) were used in the following experiments (experiments 466 and 466 A, fig. 9). The right skin glove, the control (exper. 466 A), with the *outside* of the skin turned *outward*, was filled with Ringer's solution approximately isotonic with frog's blood and was suspended alternately at 15°C. and 25°C. in the same solution. The behavior of this glove was typical of that observed in other similar experiments (fig. 2), where water was transported through the skin continuously.

The left skin glove (exper. 466, fig. 9) of the same frog, also with the *outside* of the skin turned *outward*, was filled with a 5 per cent solution of formaldehyde made up with Ringer's solution approximately isotonic with frog's blood, and was then suspended alternately at 15°C. and 25°C. in the same solution. In this case the behavior of the glove differed markedly from that of the control; the glove did not gain weight continuously, but during the first six hours of its suspension gained weight at 15°C. and lost weight at 25°C. After the sixth hour, however, a continuous, though slight, gain in weight occurred up to the twenty-third hour of the experiment.

A corresponding set of experiments was made upon the right and the left skin gloves of another frog with the *inside* of the skin turned *outward.* The right glove, the control (exper. 465 A, fig. 10), with the *inside* of the skin turned *outward,* was filled with Ringer's solution approximately isotonic with frog's blood, and was suspended in the same solution alternately at 15°C. and 25°C. The behavior of the control was similar to that previously described as commonly occurring in experiments of this character (fig. 8, first day). After the usual initial gain in weight, which occurred during the first hour of suspension, the glove lost weight continuously by a transport of water occurring through the skin outward.



9

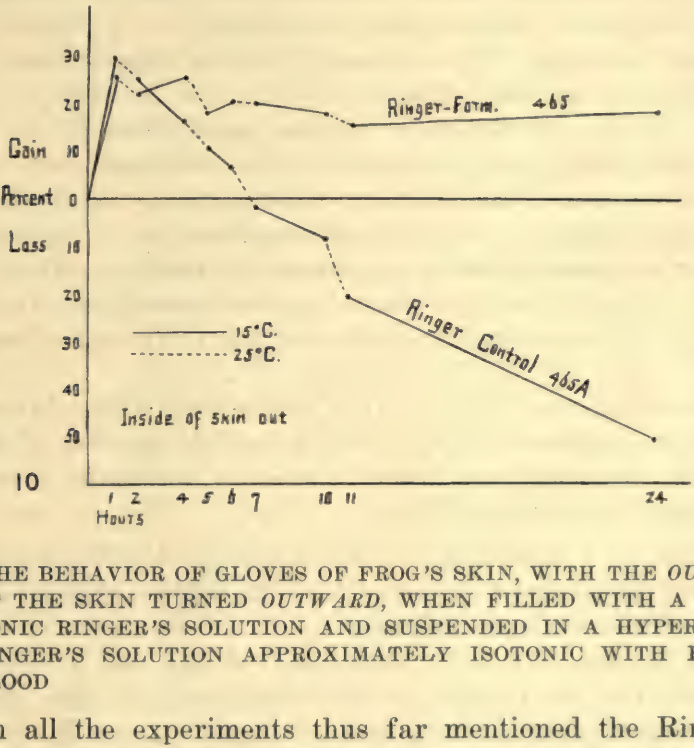
The left skin glove of the same frog (exper. 465, fig. 10), also with the *inside* of the skin turned *outward,* was filled with a 5 per cent solution of formaldehyde made up with Ringer's solution approximately isotonic with frog's blood, and was suspended in this same solution at 15°C. and 25°C. In this case, after the initial gain in weight which occurred during the first hour's suspension, the glove did not, like the control (465A), lose weight continuously when placed alternately in the solution at different temperatures; but between the second and the sixth hours of the experiment it lost weight at 25°C. and gained weight at 15°C.

While we are here undoubtedly dealing with dead skins, since the latter have been immersed in a formaldehyde solution, nevertheless the behavior of such skins emphasizes the probability that the corresponding fluctuations in weight observed in the living frog (fig. 5) and in skins removed from the body are for the most part due to the fact that the power of the skins to transport water through them continuously in opposition to osmotic pressure has been either greatly diminished or entirely destroyed. Such fluctuations in weight would therefore appear to be due to a gain or a loss of water by the skin itself. These fluctuations in weight of the skins are suggestive of the auxographic records made by MacDougal[7] on the succulent leaves of Mesenbryanthemum; these showed that the growing leaves of this plant lost water when exposed to high and gained water when exposed to low temperature.

Up to this point we have seen that the behavior of skin gloves, when filled with Ringer's solution approximately isotonic with frog's blood and suspended in the same solution, may be the resultant of the action or the interaction of several separate factors which may operate in harmony with or in opposition to one another. These factors are the power of the skin to absorb water, to release water, and to transport water through itself continuously in a direction in opposition to that of osmotic pressure. Should differences exist between the osmotic pressure of fluids which bathe the skin's opposite surfaces, the behavior of the skin glove would then be modified by osmotic pressure acting in conjunction with the above-mentioned factors. It is therefore desirable to analyze these several factors acting in conjunction with known differences in osmotic pressure.

---

[7] MacDougal, D. T. 1920 Hydration and growth. Carnegie Institution of Washington.



MARINE BIOLOGIC LIBRA

V. THE BEHAVIOR OF GLOVES OF FROG'S SKIN, WITH THE *OUTSIDE* OF THE SKIN TURNED *OUTWARD*, WHEN FILLED WITH A HYPOTONIC RINGER'S SOLUTION AND SUSPENDED IN A HYPERTONIC RINGER'S SOLUTION APPROXIMATELY ISOTONIC WITH FROG'S BLOOD

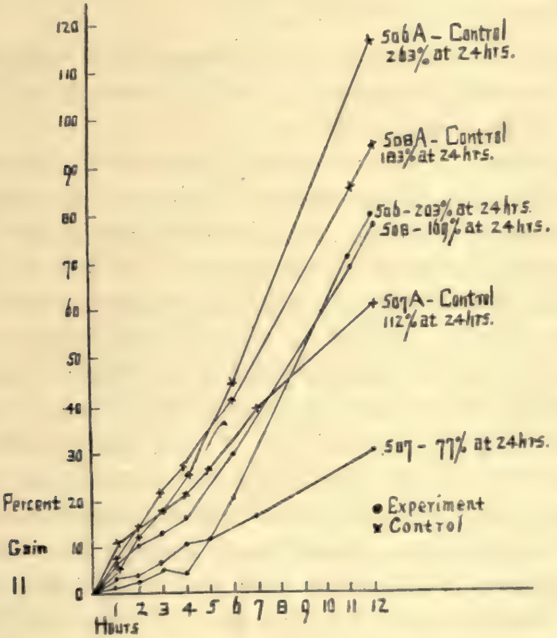
In all the experiments thus far mentioned the Ringer's solution designated as being approximately isotonic with frog's blood consisted of 7 grams of NaCl, 0.3 gram of KCl, 0.25 gram of $CaCl_2$, and 0.03 gram of $NaHCO_3$ added to 1000 cc. of distilled H_2O. This is the formula given by Howell; in the following experiments it is referred to as the isotonic Ringer's solution. In the experiments about to be mentioned, in addition to this isotonic solution, there were used three other Ringer solutions which differ from the isotonic solution only in the amount of NaCl present. As compared with the isotonic solution, these three solutions are hypotonic and contain, respectively, 6.5, 6.25, and 6 grams of NaCl, added to 1000 cc. of H_2O instead of 7 grams, as in the isotonic solution.

The following three sets of experiments (figs. 11, 12, and 13) illustrate the behavior of gloves of frog's skin, with the *outside* of the skin turned *outward,* when filled with a hypotonic Ringer's solution and suspended in a hypertonic Ringer's solution. The crosses (x) in the figures represent the behavior of the *controls;* that is, the behavior of the gloves filled with Ringer's solution approximately isotonic with frog's blood and suspended in the same solution. The circles represent the behavior of skin gloves filled with a *hypotonic* Ringer's solution and suspended in a *hypertonic* Ringer's solution which is approximately isotonic with frog's blood. In each case the gloves for the control and for the experiment were taken from the hind legs of the same frog.

1. *The behavior of gloves of frog's skin filled with a Ringer's solution containing 6.5 grams of NaCl to 1000 cc. of H_2O, when suspended in a Ringer's solution containing 7 grams of NaCl to 1000 cc. of H_2O*

Controls: The behavior of skin gloves filled with a Ringer's solution containing 7 grams of NaCl to 1000 cc. of H_2O, when suspended in the same solution.

The important feature illustrated by this set of experiments is that, as in the case of the controls, the skin gloves filled with hypotonic Ringer immediately gained in weight when placed in a hypertonic Ringer's solution. At the end of twelve hours' suspension in the solution, however, the amount gained by the gloves containing the hypotonic solution (experiments 506, 507, and 508, fig. 11) was less than that gained by the controls (experiments 506 A, 507 A, and 508 A, fig. 11), in which the gloves containing Ringer's solution approximately isotonic with frog's blood were suspended in the same solution. The fact that the rate at which water was transported through the skin into the glove was less in the experiments than in the controls indicates that in the experiments some factor must have been acting in opposition to osmotic pressure by which, until an equilibrium had been established, water should have been driven outward through the skin from the glove. This factor, as we have previously demonstrated, is the force by which water is driven through the skin in a direction opposite to that of osmotic pressure; and in the experiments this driving force exceeds that of the driving force of osmosis. We may thus account for the fact that skin gloves filled with a hypotonic Ringer's solution may gain weight when placed in a hypertonic Ringer's solution.



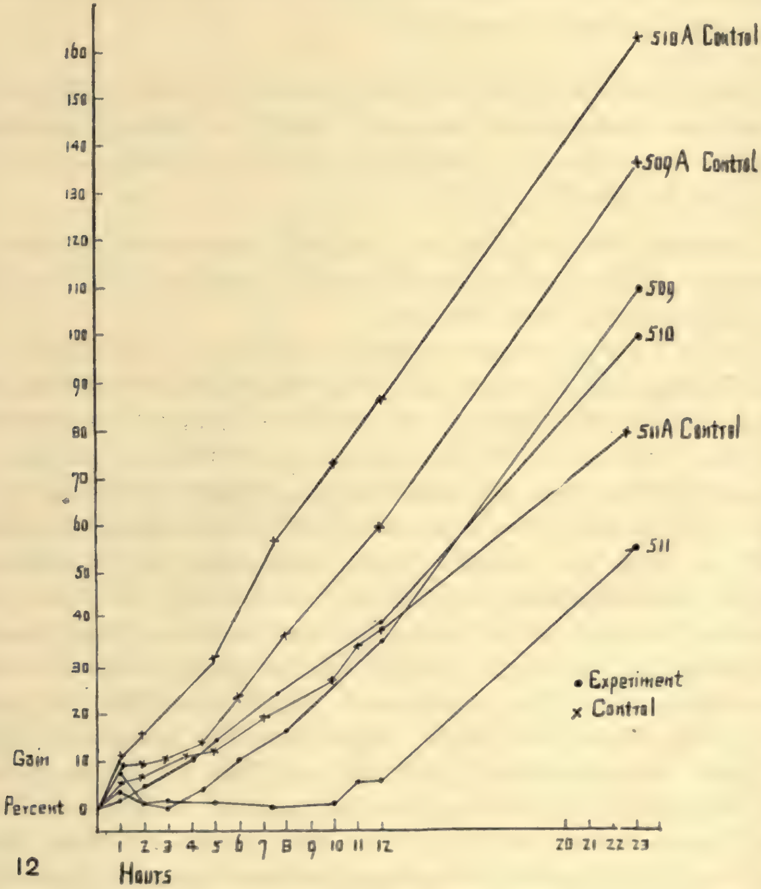
Another factor which may occasionally modify the behavior of skin gloves, under the conditions of the experiment above mentioned, is the capacity of the skin itself to gain or to lose water in conjunction with, but independently of, the forces by which water is driven inward or outward through the integument.

In experiment 506 (fig. 11) the temporary loss in weight of the glove between the third and the fourth hour is apparently due to a temporary loss of water by the skin itself.

2. The behavior of gloves of frog's skin filled with a Ringer's solution containing 6.25 grams of NaCl to 1000 cc. of H_2O, when suspended in a Ringer's solution containing 7 grams of NaCl to 1000 cc. of H_2O

Controls: The behavior of skin gloves filled with a Ringer's solution containing 7 grams of NaCl to 1000 cc. of H_2O, when suspended in the same solution

A still greater difference exists in the three following than in the last-mentioned experiments, between the osmotic pressure of the Ringer solution that is contained in the glove and that in which the glove is suspended. It will be observed that the controls of these three experiments (509 A, 510 A, and 511 A, fig. 12) gained in weight continuously from the time the gloves were first suspended in Ringer, and that the amount gained at the end of twenty-three hours' suspension was greatest in the control 510 A and least in 511 A. In experiment 510, of which 510 A is the control, the glove containing hypotonic Ringer gained weight continuously for a period of twenty-three hours and, except that the amount gained was less, its behavior was similar to that of its control. The circumstance that the gain in weight of this glove (510) containing hypotonic Ringer was continuous throughout the experiment might be explained by the fact that this particular skin may have possessed marked capacity to transport water through itself in a direction opposite to the force of osmotic pressure and at a rate which at all times exceeded that at which water was driven by osmosis through the skin outward. On the other hand, there is a marked difference between the behavior of the two remaining gloves and that observed in experiment 510 and in the three controls. In both cases in which the glove containing hypotonic Ringer was suspended in hypertonic Ringer, the glove gained in weight during the first hour of its suspension (experiments 509 and 511). This gain in weight, however, was succeeded by a loss, which was followed by a gain that continued through the remaining period of the experiment. It is evident that the initial and temporary gain in weight observed in experiments 509 and 511 could not be accounted for on the grounds of differences in osmotic pressure, but was more probably due to the presence of water absorbed by the skin itself. This temporary gain followed by a temporary loss in



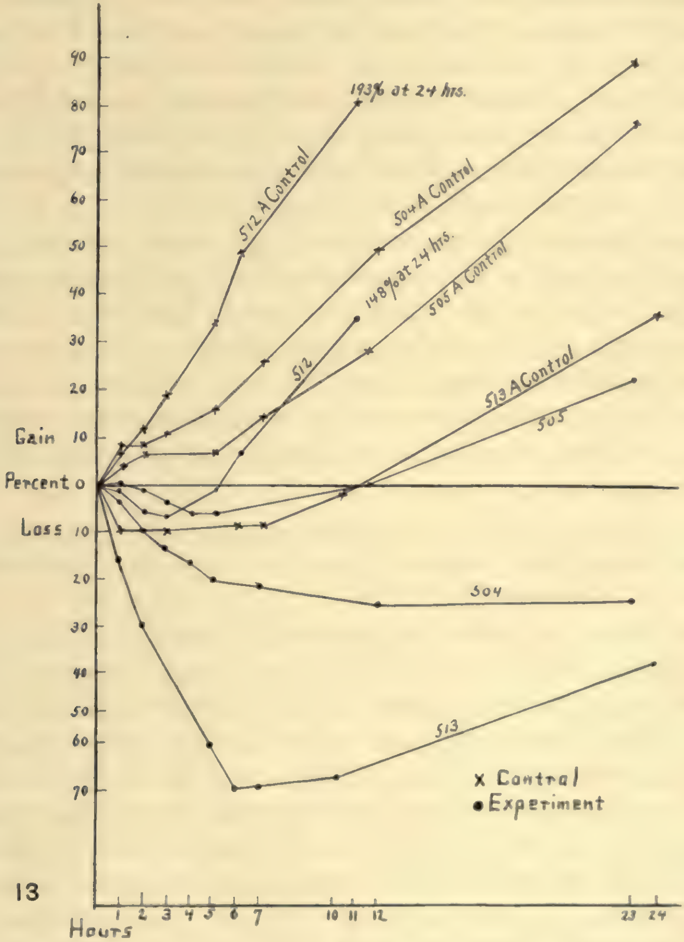
12

weight suggests that, after the hydration capacity of the skin had been satisfied, there was set up an osmotic transfer which continued either until an equilibrium had been established or until the driving force of osmosis was exceeded by the force which drives water through the skin in the opposite direction to osmotic pressure.

3. The behavior of gloves of frog's skin filled with a Ringer's solution containing 6 grams of NaCl to 1000 cc. of H_2O, when suspended in a Ringer's solution containing 7 grams of NaCl to 1000 cc. of H_2O

Controls: The behavior of skin gloves filled with a Ringer's solution containing 7 grams of NaCl to 1000 cc. of H_2O, when suspended in the same solution.

This set of experiments clearly illustrates the effects of the driving force of osmosis when this force is sufficiently great to overcome that force by which water is driven through the skin into the glove in opposition to osmotic pressure. In each of the four experiments graphically represented in figure 13, the skin glove containing a hypotonic Ringer's solution at first lost weight when suspended in hypertonic Ringer (experiments 504, 505, 512, and 513). In three instances, however, after an equilibrium had been established, the gloves began to gain weight and continued to do so until the termination of the experiment (experiments 505, 512, and 513). In this latter respect their behavior resembled that of their controls (505 A, 512 A, and 513 A) which, from the beginning of their suspension, gained weight continuously under conditions in which both surfaces of the skin were bathed by solutions having the same osmotic pressure. In the control 513 A the glove lost weight during the first hour of its suspension in Ringer; its weight then remained practically stationary for about six hours, after which time, however, there came a gain in weight. This form of behavior has already been demonstrated for certain skins in which the capacity to transport water through the skin in a direction opposite to that of osmotic pressure has been either greatly diminished or entirely lost (exper. 442 A, fig. 3). The initial loss of water by the glove in control 513 A can be ascribed not to differences in osmotic pressure of the fluids which bathe the skin's opposite surfaces, but rather to a loss by



13

the skin itself of water which it had previously held. Also, it may be observed that in experiment 513 the initial loss in the weight of the glove greatly exceeds that observed in the gloves in the three other experiments (experiments 504, 505, and 512) as well as that in its control (513 A). It is evident that we may account for the greater loss in weight of the glove in experiment 513 on the ground that in this particular skin, as in that of its control (513 A), there was acting in opposition to osmotic pressure no force by which water could be simultaneously driven through the skin into the glove.

It would appear that the behavior manifested by skin gloves, with the outside of the skin turned outward, when filled with a hypotonic Ringer's solution and suspended in a hypertonic Ringer's solution, may be the resultant of the action and interaction of the following separate and distinct factors: osmotic pressure; the capacity of the skin itself to gain and to lose water; and a force which drives water through the skin in a direction opposite to that of osmotic pressure. Also, we find that skin gloves filled with a hypotonic solution and suspended in a hypertonic solution begin to gain weight after an equilibrium has been established, in proportion to the capacity of the skin to transport water through itself when both its surfaces are bathed by solutions having the same osmotic pressure.

VI. ON THE TRANSPORT OF WATER THROUGH THE SKIN IN THE LIVING FROG WITH URETERS LIGATED, WHEN SUSPENDED IN A HYPERTONIC SALINE SOLUTION

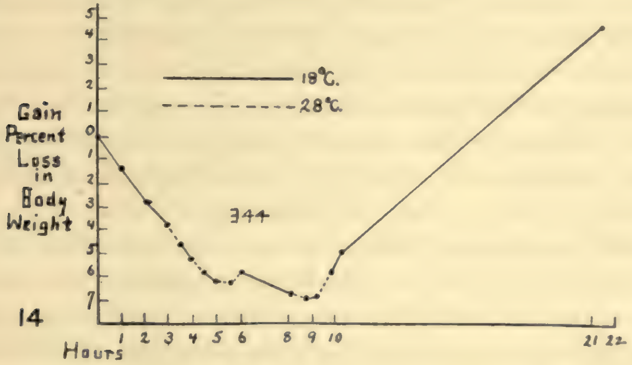
It has been shown by the writer in a former paper ('19) that when the ureters have been ligated and a frog or toad is placed in water, there immediately follows a gain in body weight which may gradually result in an intense form of generalized oedema. These experiments should not be confused with those of Overton ('04), who ligated the cloacal opening, but not the ureters. When only the cloacal opening is ligated, the flow of urine through the ureters into the bladder and cloaca is not interrupted. In such cases, as stated by Overton, the bladder becomes filled with urine which backs up into the digestive canal. After the cloacal ligature is removed, this urine is readily expressed from the bladder and intestinal canal. On the other hand, after the ligature has been removed from the ureters, ordinarily no fluid can immediately be expressed from the cloaca, as in this case the excess of fluid contained in the body has been deposited as lymph in the tissues and cavities of the body, and not as urine in the cloaca and intestinal canal. Therefore, only by ligating the ureters can a typical oedematous condition of the body be produced.

It has previously been observed by a number of writers that living frogs, as well as toads, lose weight when suspended in hypertonic saline solutions. We are now in a position to compare the behavior of a living frog whose ureters have been ligated with that of a skin glove filled with Ringer's solution isotonic with frog's blood, when each is suspended in a hypertonic solution. In the case of the living frog, with ureters ligated, the conditions are essentially analogous to those in the skin glove; each consists of a closed sac of frog's skin. In the experiment about to be mentioned and in others similar, the cloacal opening was ligated in addition to the ureters. This was done to insure against the fluid's escaping from the body in case an extravasation should occur at the point where the ureters had been ligated. The ligation was accomplished by passing under the ureters a silk ligature which was tied over the urostyle. The cloacal opening also was tied with a silk ligature. It is no easy matter in all cases to get what one may regard as a satisfactory ligation; injury to the skin by the needle is very likely to permit lymph in the dorsal subcutaneous lymph sinuses to extravasate through the openings. Unless the ligation is practically perfect, the frog or toad should not be used for experiments of this character. Better results have usually been obtained on frog's recently caught than on those which have remained for some time in the laboratory. This seems to be due to the fact that in pale-skinned winter frogs the skin is much thinner and is more easily injured than in those recently captured. On account of the toughness of their skin, toads have generally been found to be more favorable subjects for these experiments than are frogs.

In the following experiment (344, fig. 14) a living frog, with its ureters and cloacal opening ligated, was fastened to a frog board and was alternately suspended up to the armpits in a 0.9 per cent solution of NaCl at 18°C. and 28°C. The frog (R. pipiens) was obtained from a dealer on September 25th. The experiment, beginning on September 26th, extended over a period of about twenty-one hours, during which time the frog was suspended in a hypertonic saline solution. When a ligated frog is suspended in a solution in this manner, it is evident that no fluid can possibly enter or leave the body except by passing through the integument. When frogs are placed in finger-bowls containing a saline solution, fluid is often taken into the intestinal canal through the oral cavity; hence the method of suspending them on frog boards is essential to this type of experiment.

It will be observed that during the first nine hours of its suspension in the hypertonic solution (fig. 14), the ligated frog lost weight continuously, except during the sixth hour, when there was a slight gain. With the tenth hour, however, the frog began to gain in weight, and continued to do so until the close of the experiment. It is therefore seen that the behavior of the living frog with ureters ligated when suspended in a hypertonic saline solution (fig. 14) is identical with that of a skin glove, with the outside of the skin turned outward, when filled with Ringer's solution and suspended in a hypertonic Ringer's solution (fig. 13). It is evident that the loss in weight which immediately follows the suspension of the living frog in the hypertonic solution is, as in the case of the skin glove, due chiefly to differences in the osmotic pressure of the fluids which bathe the skin's opposite surfaces. Also, in each instance, after an equilibrium has been established and water is no longer driven by osmosis through the skin outward, a gain in weight of the frog and of the skin glove follows, due to the existence of a force which drives water inward through the skin in a direction opposite to that of the driving force of osmosis.

We know that when the living frog remains in water, water is driven by osmosis through the skin into the subcutaneous lymph sinuses, and that the amount of water supplied in this manner to the body is influenced by temperature. In view of the observations made in the preceding pages, we have reason to believe that in the living frog, in addition to, though in conjunction with this osmotic transfer, water is also driven through the skin into the subcutaneous lymph sinuses by a force acting independently of osmotic pressure, so that the water supply to the body is not obtained exclusively from that driven through the skin by osmosis. Furthermore, during the transport of water through the skin by either method, the skin itself may absorb or it may lose water. Should we therefore attempt to obtain the temperature coefficient of absorption of the skin, either in a skin glove or in the living animal, we should expect this coefficient to vary in relation to the capacity of any particular skin to transfer water through itself independently of the osmotic transfer, as well as to absorb or to lose water. A coefficient of absorption of the living skin should therefore be the resultant of the action and interaction of a number of factors which include osmotic pressure, a force which drives water through the skin independently of osmotic pressure, and a gain or loss of water by the tissue colloids.



VII. ON THE NATURE OF THE FORCE WHICH MAY DRIVE WATER THROUGH THE SKIN IN A DIRECTION COUNTER TO THAT CAUSED BY OSMOTIC PRESSURE

We have shown in the preceding pages that a number of factors may be concerned in the transport of water through the skin in the living frog, as well as through the skin when detached from the body. These factors include osmotic pressure, the capacity of the skin itself to absorb and to lose water, and a force which acts simultaneously and in conjunction with osmotic pressure, though independently of this. Having demonstrated the rôle played by each of these factors when acting either independently or in conjunction with one another, we must now analyze more closely the nature of the force which is able to drive water through the skin in a direction opposite to that of osmotic pressure.

In 1918, Gunzberg[8] published a paper dealing with the pathology of oedema, in which he advanced the view that the older theories of filtration, osmosis, and the affinity of colloids for water, do not afford an adequate explanation of oedema, and that the passage of water into the intercellular spaces is due to the electrical properties of the membrane separating circulating fluids from the tissues. He stated that the E. M. F. is due to membrane polarization; that the element K and the ions H and OH play an important rôle in this electrical osmosis, which is able to drive the fluid in a direction opposite to that of osmotic pressure. Gunzberg's view was based on experiments made on pithed frogs which were perfused with Ringer's solution and modifications of this solution; in these experiments the fluid entered by a canula in the aorta and left by the proximal end of this vessel. Gunzberg found that careful perfusion with a 0.7 per cent NaCl or with Ringer's solution caused no oedema. When, however, the Ringer's solution was deprived of K, there resulted a characteristic oedema in which the final weight showed as much as a 40 per cent increase in the course of twenty-four hours. In such cases the lymph spaces and the pleuro-abdominal cavity were filled with fluid, and the stomach, bladder, and intestines produced an abundant secretion. Gunzberg found also that skin gloves filled with Ringer and immersed in a liquid with an excess or absence of K, increased in weight, while the weight of a glove filled with Ringer minus K and immersed in Ringer diminished.

---

[8] Gunzberg, I. 1918 Contribution a l'étude de pathogenie de l'oedeme. Archiv. neer. de Physiol., vol. 2.

In the light of the experiments described in the preceding pages, the writer has made a detailed study of the results obtained by Gunzberg. The results obtained by him would seem to indicate that the force by which fluid is transported through the skin, independently of osmotic pressure, may be regarded in the light of an electrical phenomenon.

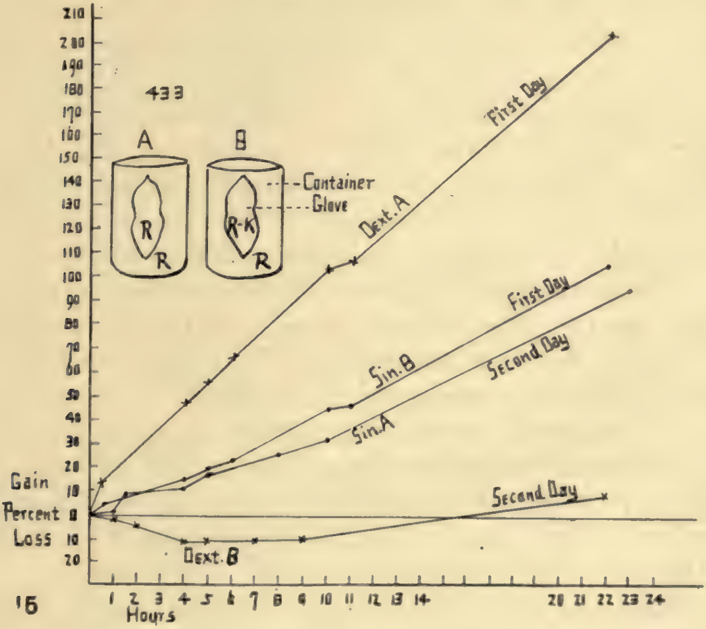
A number of experiments were made on skin gloves, with the outside of the skin turned outward, as well as inward, to determine the rate of transport of fluid through the skin, in relation to the presence or absence of K both in the Ringer's solution contained in the glove and also in the solution in which the glove was suspended. Skin gloves removed from the hind legs were used; the glove from the right leg was, as a rule, used for the control and the glove from the left leg of the same frog was used for the experiment. Also, as in all of the preceding experiments, the gain or the loss in weight of the glove was based on the original weight of the empty skin glove, before fluid was placed in the glove, and prior to the suspension of the glove in the solution. The results of these experiments are graphically shown in figures 15 to 23, inclusive. The variability observed in the behavior of different skins in the same type of experiment, in relation to the rate at which fluid is transported through the skin independently of osmotic pressure, is analogous to that previously described as commonly existing among skins obtained from different animals (figs. 1 to 4, inclusive). A knowledge of this variability for skins in general has therefore made possible a correct interpretation of the variable conditions observed in these experiments.

First, experiments were made to determine what rôle the element K generally plays in influencing the velocity of diffusion of water through frog skins taken from different animals of the same and of different species. After this had been determined, special type experiments were made on skin gloves, with the outside of the skin turned outward as well as inward, in which the conditions were modified in various ways, as regards the presence and absence of K in the Ringer's solution contained in the glove and in the solution in which the glove was suspended.

Experiment 433 (fig. 15)

The right and the left skin gloves of the same frog (R. pipiens), with the *outside* of the skin turned *outward,* were first filled, respectively, with Ringer's solution approximately isotonic with frog's blood and with Ringer minus K, and were then suspended in Ringer's solution. (Diagrams A and B in fig. 15 illustrate the type of the experiment, as regards the character of the Ringer's solution in the glove and that of the Ringer in the container in which the glove was suspended.) During the course of twenty-two hours' suspension, the right glove, which contained Ringer, and which in this instance served as the control (*Dext. A, first day*), gained more in weight than did the left glove which contained Ringer minus K (*Sin. B, first day,* fig. 15). At the end of twenty-two hours, the right glove was filled with Ringer minus K, instead of Ringer (*Dext. B, second day*), and the left glove with Ringer, instead of Ringer minus K (*Sin. A, second day,* fig. 15), and again both gloves were suspended in Ringer for another period of twenty-two hours. Figure 15 shows graphically that, during the second day's suspension, the left glove containing Ringer in which K was present (*Sin. A, second day*) gained more in weight than the right glove in which K was absent (*Dext. B, second day*). The behavior of the control on the first day (*Dext. A, first day*) signifies a marked capacity of the skin of this particular frog to transport water through itself under conditions in which both surfaces of the skin are bathed by solutions having the same osmotic pressure.

One often finds skins through which, soon after their removal from the frog, fluid does not immediately diffuse when they are filled with Ringer's solution isotonic with frog's blood and are suspended in the same solution (fig. 3). In such cases it has been found that the presence or absence of K in the Ringer's solution contained in the glove does not influence the behavior. After a more or less prolonged suspension of the glove in Ringer, however, a transport of fluid through the skin is usually initiated, and the weight of the glove containing Ringer in which K is present then increases at a greater rate than that of the glove containing Ringer in which K is absent. In other words, only in the case of skins in which a transfer of fluid is actually being accomplished is the behavior of the glove influenced by the presence or absence of K in the solutions. An example of this form of behavior is illustrated by the following experiment.



Experiment 429 (fig. 16)

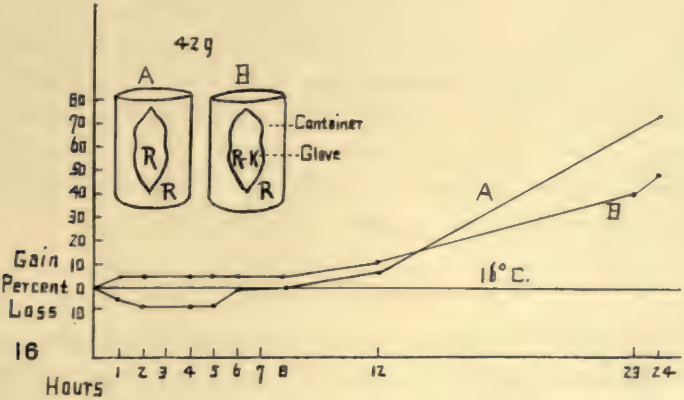
The right and the left skin gloves of the same frog (R. pipiens), with the *outside* of the skin turned *outward,* were filled, respectively, with Ringer and Ringer minus K and were then suspended in Ringer's solution. (As in fig. 15, diagrams A and B indicate the type of experiment.) The behavior of the gloves showed that in neither case was there a continuous transfer of fluid through the skin during the first eight hours' suspension in Ringer. From the eighth hour on, however, water began to diffuse through the skin into the gloves, and a gradual gain in weight of the gloves followed. At the end of twenty-three hours' suspension, the weight of the glove containing Ringer in which K was present (A, fig. 16) was greater than that of the glove in which K was absent (B, fig. 16). Why, under the conditions of the experiment, water should not be transported through the skin at the time of its removal from the body, but should be later on, has not up to the present time been determined. Such is the case, however, and it is evident that the recognition of this fact explains the aberrant behavior of the gloves in question.

The writer made sixteen preliminary experiments of the type illustrated by figures 15 and 16, in all of which the results were essentially uniform, in the sense that they conformed with those of the experiments mentioned by showing that the ions of K influence the velocity of diffusion.

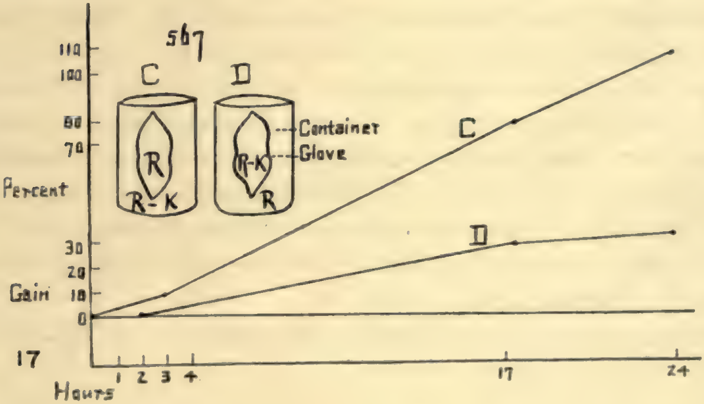
We may now proceed to the consideration of a series of experiments based upon those above mentioned, in which the conditions have been still further modified.

Experiment 567 (fig. 17)

Two skin gloves removed from the hind legs of the same frog (R. pipiens), with the *outside* of the skin turned *outward,* were filled, one with Ringer and the other with Ringer minus K, and were then suspended, respectively, in Ringer minus K and in Ringer. (See diagrams C and D in fig. 17 for type of experiment.) Four experiments of this character were made, in three of which the behavior observed was essentially similar to that graphically shown in figure 17, in which it is seen that during the course of twenty-four hours the glove containing Ringer (C) gained more in weight than the glove containing Ringer in which K was absent (D). In the other case, however, as in experiment 429 (fig. 16), a transfer of water did not take place in either skin until after the gloves had been for some time suspended in the solutions. After a transfer had been initiated, however, the glove containing Ringer, in which K was present, began to gain weight in excess of the glove containing Ringer in which K was absent.



16



17

It has previously been demonstrated (fig. 6) that, when the *inside* of the skin is turned *outward,* the direction in which fluid is transported through the skin is exactly opposite to that in which fluid is transported when the *outside* of the skin is turned *outward.* If the presence of K in the Ringer's solution influences the rate at which fluid is transported inward through the skin into the glove, when the outside of the skin is turned outward, it is evident that the rate at which fluid is transported outward through the skin from the glove when the inside of the skin is turned outward should be similarly influenced. Such has been found to be the case, as is shown by the following experiments which are of the same type as the preceding experiment (exper. 567, fig. 17).

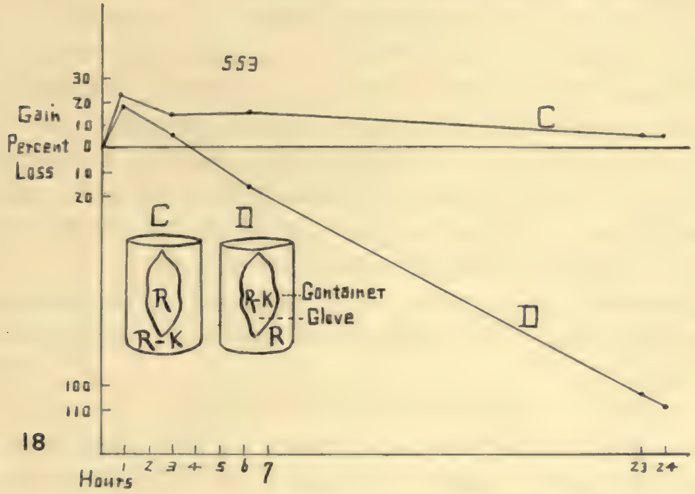
Experiment 553 (fig. 18)

Two skin gloves removed from the hind legs of the same frog (R. pipiens), with the *inside* of the skin turned *outward,* were filled, one with Ringer and the other with Ringer minus K, and were then suspended, respectively, in Ringer minus K and Ringer. (For type of experiment see diagrams C and D in fig. 18.) Four experiments of this character were made, and in each instance, after the usual initial gain in weight during the first hour's suspension, the glove containing Ringer minus K (D in fig. 18), when suspended in Ringer, lost more in weight during the course of twenty-four hours than did the glove containing Ringer when suspended in Ringer minus K (C in fig. 18).

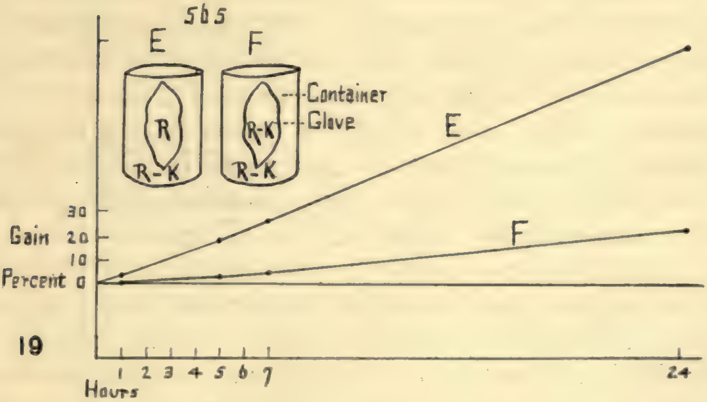
Another type of experiment which differs from that of the preceding experiments follows.

Experiment 565 (fig. 19)

The right and the left skin gloves of the same frog (R. pipiens), with the *outside* of the skin turned *outward,* were filled, one with Ringer and the other with Ringer minus K, and were then suspended in Ringer minus K. (See diagrams E and F in fig. 19, which indicate type of experiment.) Four experiments of this character were made. In two instances the behavior of the gloves was similar to that graphically represented in figure 19, in which the glove containing Ringer (E, fig. 19) gained more in weight during the course of twenty-four hours than did the glove containing Ringer in which K was absent (F, fig. 19). In the two other experiments the presence or absence of K in the solutions did not, during the first six hours of their suspension, influence the behavior of the gloves. Later on, however, the glove containing Ringer in which



18



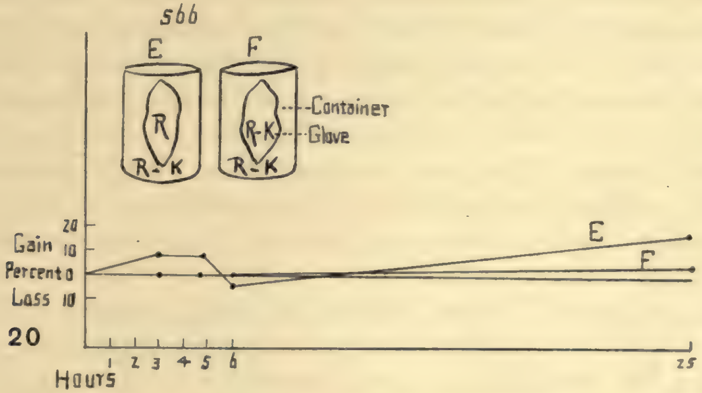
19

K was present began to gain weight in excess of the glove containing Ringer in which K was absent. This form of behavior, as previously shown (fig. 16), is characteristic of certain skins through which, at the time of their removal from the body, water does not immediately begin to diffuse when both sides of the skin are bathed by solutions having the same osmotic pressure. In one of these two experiments the initial gain followed by the loss in weight (E, fig. 20) observed during the first six hours of its suspension, is probably due to the absorption and the loss of water by the skin itself, and not to a transport of fluid through the skin into the glove during the first three hours, and a transport from the glove through the skin outward in the fifth and sixth hours.

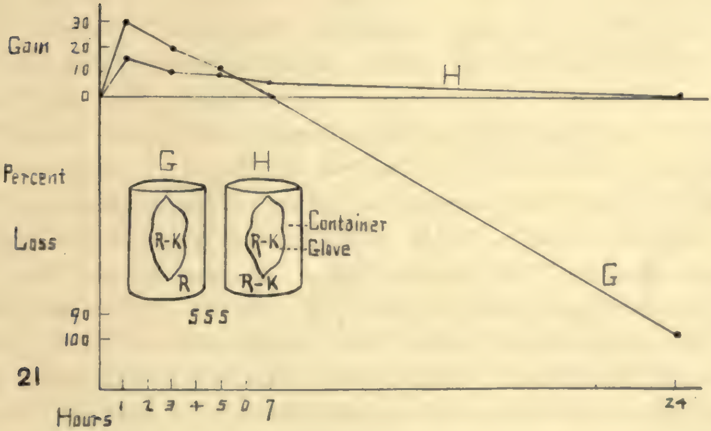
Eight more experiments of still another type were made on skin gloves in which the *inside* of the skin was turned *outward.*

Experiment 555 (fig. 21)

The right and the left skin gloves of the same frog (R. pipiens), with the *inside* of the skin turned *outward,* were filled with Ringer minus K and were suspended, respectively, in Ringer and Ringer minus K. (For type of experiment see diagrams G and H in fig. 21.) In five of these experiments the behavior of the gloves was essentially similar to that graphically shown in figure 21, in which, during the course of twenty-four hours, the glove suspended in Ringer (G) lost more in weight than did the glove containing Ringer minus K when suspended in Ringer in which K was absent (H, fig. 21). In each instance a loss in weight of the glove was preceded by a gain, which occurred during the first hour's suspension. This gain in weight, we have seen, is probably due to an addition of water to the skin itself, through imbibition. In three of these experiments a transfer of water through the skin did not take place until after the gloves had been suspended for several hours in the solutions. After the transfer had been established, however, the rate of diffusion was found to be influenced by the presence of K in the solution in which the gloves were suspended. When we compare the behavior observed in experiment 555 with that observed in the preceding experiments, it becomes evident that the presence of K accelerates the rate of diffusion of water through the skin in that particular direction through which, under conditions of the experiment, a diffusion is normally accomplished.



20



21

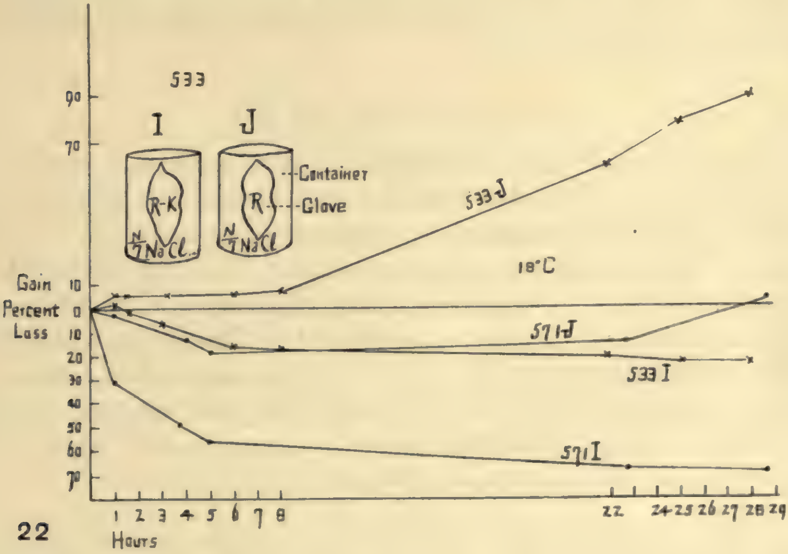
We have previously shown that the force which may drive water through the skin into the glove, when both surfaces of the skin are bathed by solutions having the same osmotic pressure, may also drive water through the skin when skin gloves containing Ringer are suspended in a hypertonic

Ringer's solution (figs. 11, 12, and 13). In the following experiments we show how the action of this force may be modified by the presence or absence of K in the Ringer's solution.

Experiments 533 and 571 (fig. 22)

The right and the left skin gloves of the same frog (R. pipiens with the *outside* of the skin turned *outward,* were filled, respectively, with Ringer minus K and Ringer, both approximately isotonic with frog's blood, and were then suspended in a hypertonic saline solution (N/7 NaCl). (See diagrams I and J in fig. 22 for type of experiment.) Six experiments of this character were made; the general form of behavior manifested by the gloves in two of these experiments is graphically shown in figure 22.

In experiment 571 both gloves lost weight during the first five hours of their suspension in the hypertonic solution, and in the glove containing Ringer in which K was absent (571 I) the loss was greater than in the glove in which K was present (571 J). While, in each instance, this loss resulted from differences in osmotic pressure, the relatively slight loss in weight of one of the gloves (571 J) indicates that the force which drives water through the skin into the glove, in the opposite direction to that by which it is driven by osmosis, was greater in one skin than in the other. This increase in the rate of passage, due to the presence of K in the solution, counteracted the full driving force of osmosis, so that the glove containing Ringer in which K was present (571 J) lost less water and consequently less weight than did the glove containing Ringer in which K was absent (571 I) when the gloves were suspended in the hypertonic saline solution. From the fifth hour on, the glove containing Ringer in which K was present (571 J) gradually made a gain which amounted to about 92.5 per cent at the end of forty-six hours' suspension in the hypertonic solution. This gradual gain indicates that after an equilibrium had been established between the Ringer's solution in the glove and the NaCl solution in which the glove was suspended, water was driven through the skin into the glove by some force other than osmotic pressure and that the velocity of the transfer was increased by the presence of K in the Ringer's solution. On the other hand, after the fifth hour of suspension the behavior of the glove containing Ringer in which K was absent (571 I) differed somewhat from that of the glove containing Ringer in which K was present (571 J). In this instance an equilibrium had been established sometime between the fifth and the twenty-second hour; the weight of the glove then remained stationary for a considerable time, after which, at the end of forty-eight hours' suspension in the hypertonic solution, there followed a slight gain in weight amounting to about 13 per cent.



22

In relation to the presence and absence of K in the Ringer's solution, the general behavior of the gloves in experiment 533 (fig. 22) is the same as in experiment 571 (fig. 22). The fact that the initial loss in weight of the glove in experiment 571 was greater than in experiment 533 may be explained on the ground that the capacity of the skin to transfer water through itself in a direction opposite to that of osmotic pressure was less in experiment 571 than in experiment 533.

In the above experiments we have shown how the driving force of osmosis may be modified by the presence of K in the Ringer's solution contained in the glove when the latter, with the *outside* of the skin turned *outward*, is suspended in a N/7 NaCl solution. Let us now reverse the conditions and study the behavior of gloves with the *inside* of the skin turned *outward,* when filled with N/7 NaCl and suspended, respectively, in Ringer minus K and in Ringer. As in the other experiments, the Ringer's solution used was approximately isotonic with frog's blood.

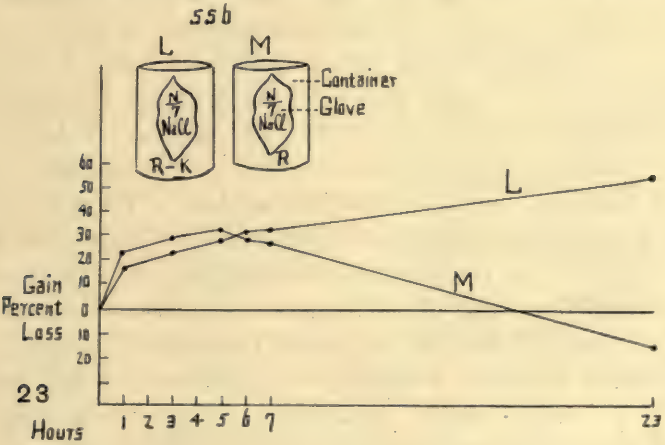
Experiment 556 (fig. 23)

Four experiments of this character were made and, in three instances, the behavior observed was essentially similar to that of the gloves graphically represented in figure 23. (For type of experiment see diagrams L and M in fig. 23.) The essential feature to be noted is that after an equilibrium had been established, the glove suspended in Ringer in which K was present (M) began to lose weight, on account of a transfer of water from the glove through the skin outward—that is, in the normal direction taken by the transfer when the *inside* of the skin is turned *outward.*

VIII. ON A POSSIBLE EXPLANATION OF THE CONTINUOUS FLOW OF LYMPH BETWEEN THE CAPILLARIES OF THE BLOOD-VASCULAR SYSTEM AND THOSE OF THE BLINDLY-ENDING LYMPHATICS

The results of the experiments detailed in the preceding pages lead us to the conclusion that when the living frog remains in water, three sets of factors may be involved in the transportation of fluid through the skin into the subcutaneous lymph sinuses. These factors constitute, 1) the varying hydration capacity of the tissue colloids by which the skin itself may absorb or lose water; 2) osmotic pressure, and, 3) a force, electrical osmosis, which acts independently of, and simultaneously with osmotic pressure, in driving water through the skin into the subcutaneous lymph sinuses.

After fluid has been transported through the skin into the subcutaneous lymph sinuses, it is then conveyed from the latter in the form of lymph to the blood-vascular system, where it mingles with the blood plasma. Some of this blood plasma is then transported in the form of lymph through the walls of the blood capillaries into the tissue spaces, from which it may be taken up and held by the tissue colloids and be collected by the blindly-ending lymphatics which return it to the blood-vascular system. In other words, when the living frog remains in water, a continuous flow of fluid is taking place through the skin into the subcutaneous lymph sinuses, and thence through the blood-vascular system and tissue spaces back again into the lymphatic system.



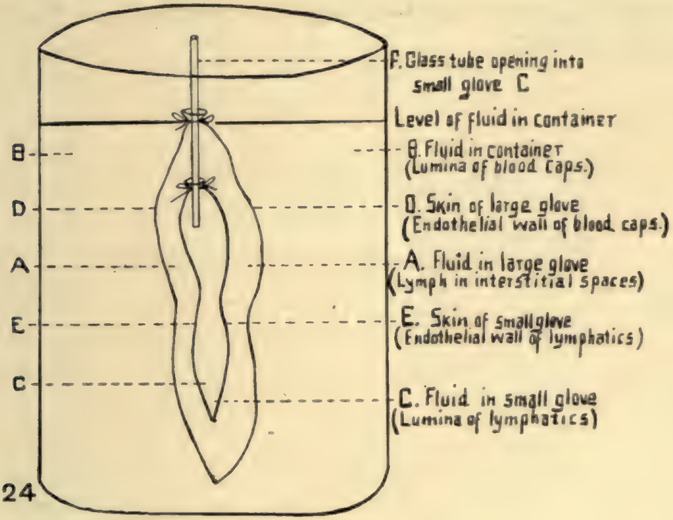
23

On the assumption that the same factors are involved in the transportation of fluid through the endothelial walls of the capillaries of the blood-vascular and lymphatic systems, as through the skin into the subcutaneous lymph sinuses, we have at least a working hypothesis to account for the establishment and maintenance of a continuous flow of fluid between the blood capillaries and those of the blindly-ending lymphatics. In any event, on this assumption, we can demonstrate a somewhat analogous condition by the following experiment which is based on the results of experiments mentioned in the preceding pages.

If a large glove of frog's skin (A, fig. 24), with the *outside* of the skin turned *outward,* is filled with Ringer's solution isotonic with frog's blood and is then suspended in a container (B) filled with the same solution, we have seen that fluid can be transported continuously from the container through the skin (D) into the glove (A). If a smaller glove (C, fig. 24), with the *outside* of the skin turned *outward,* is now filled with isotonic Ringer, and is placed inside the larger glove (A, fig. 24), water will be transported continuously from the large glove (A) through the skin into the smaller glove (C). Now, with both surfaces of both skin gloves bathed by solutions having the same osmotic pressure, we may expect to establish a continuous flow of fluid passing from the container (B) through both skins (D and E) into the smaller glove (C). Any excess of fluid in the smaller glove (C) would flow from the latter into the capillary tube (F).

If, in either of the two gloves, we should substitute for the Ringer's solution isotonic with frog's blood a Ringer solution having a slightly lower osmotic pressure, we know that water will continue to be transported through the skin in the same manner and direction. If a Ringer's solution containing 6.5 grams of NaCl to 1000 cc. of water, instead of 7 grams as in the so-called isotonic solution, were placed in the smaller glove (C), water would still continue to be transported through the skin from the large into the smaller glove, in the opposite direction to osmotic pressure. (See experiments 506, 507, and 508, fig. 11.) Also, if this same hypotonic Ringer solution were substituted for the isotonic solution in the larger glove (A), water in container B would still continue to be transported in a continuous manner through the skin into the larger glove and through the skin into the smaller one (C), even though the latter contained a Ringer solution of the same or of a slightly higher concentration than that in the larger glove. Also, it is evident that the rate at which fluid would be transported through the skin might be modified by the presence or absence of K in the solutions. (See experiments illustrated by figs. 15 to 23, inclusive.)

If we assume that B in figure 24 represents the lumina of the blood capillaries containing blood plasma; A, the interstitial spaces, containing lymph which has diffused through the walls of the blood capillaries (D); E, the walls of the blindly-ending lymphatics, and C, the lumina of the lymphatic vessels, containing lymph which has diffused through the endothelial walls of the lymphatics (E) from the interstitial spaces (A), we can picture how, under slightly varying conditions of the electrolytes and of the concentration of the body fluids, a continuous flow of fluid might be established between the blood capillaries and the lymphatics. Also, as in the case of the frog's skin, it is quite possible that the normal physiological direction in which fluids are transported through the endothelial walls may depend upon which surface of the capillary wall is directed outward or inward, so that, if we could experimentally turn the inner surface of the lymphatic capillaries outward, lymph might then diffuse into the tissue spaces from the lymphatics.


F. Glass tube opening into small glove C
Level of fluid in container
B
B. Fluid in container (Lumina of blood caps.)
D
D. Skin of large glove (Endothelial wall of blood caps.)
A
A. Fluid in large glove (Lymph in interstitial spaces)
E
E. Skin of small glove (Endothelial wall of lymphatics)
C
C. Fluid in small glove (Lumina of lymphatics)

24

We may therefore assume the possibility that the flow of fluid between the blood capillaries and the lymphatics may be established and continued in the manner suggested. One important question still remains to be determined—whether the rate of passage through the skin, as observed in the experiments, is consistent with that which actually prevails in the living animal. Be this as it may, the significance of these observations cannot be disregarded in an attempt to account for the establishment and the continuance of a flow of fluids between the blood capillaries and the lymphatics, and for variations in the rate of flow, due to differences in potentiality which actually exist on opposite surfaces of the skin in the living animal.

The Wistar Institute of Anatomy and Biology

36th Street and Woodland Avenue :: :: Philadelphia, Pa.

The Anatomical Record

Editor

John Lewis Bremer

Harvard Medical School, 240 Longwood Ave., Boston, Massachusetts

Published Monthly
About 400 pages per volume

Price { $5.00 per volume, Domestic
$5.50 per volume, Foreign

The Journal of Experimental Zoölogy

Editorial Board

William E. Castle
Edwin G. Conklin
Charles B. Davenport
Herbert S. Jennings
Frank R. Lillie
Thomas H. Morgan
George H. Parker
Raymond Pearl
Charles R. Stockard
Edmund B. Wilson

Ross G. Harrison, *Managing Editor*

Osborn Zoölogical Laboratory, Yale University, New Haven, Connecticut

Published eight times a year
About 500 pages per volume

Price { $7.50 per volume, Domestic
$8.00 per volume, Foreign

American Journal of Physical Anthropology

Editor

Aleš Hrdlička

U. S. National Museum, Washington, D. C.

E. A. Hooton
George Grant MacCurdy
Charles B. Davenport
Franz Boas
Clark Wissler
J. Howard McGregor
Davidson Black
H. H. Donaldson
Richard E. Scammon
T. Wingate Todd
R. J. Terry
Robert Bennett Bean
Berthold Laufer
Charles H. Danforth
J. C. Merriam
A. L. Kroeber
A. E. Jenks
Adolph H. Schultz

Published Quarterly
About 500 pages per volume

Price { $6.00 per volume, Domestic
$6.50 per volume, Foreign

THE WISTAR INSTITUTE PRESS

AUTHORS'

ABSTRACTS

issued in advance

of all papers to appear in the

Journal of Morphology and Physiology
The Journal of Comparative Neurology
The American Journal of Anatomy
The Anatomical Record
The Journal of Experimental Zoology
American Anatomical Memoirs
American Journal of Physical Anthropology
Folia Anatomica Japonica (Tokyo, Japan)
Biological Bulletin
The Journal of Parasitology
Stain Technology
Australian Journal of Experimental Biology and Medical Science (Adelaide, South Australia)

will save time in following current zoological research. No paper will escape attention if you have at hand

The Wistar Institute Bibliographic Service

Advance Abstract Sheets - - - $3.00 per year
Issued every few days

Bibliographic Service Cards - - $5.00 per year
With complete bibliographic references

THE WISTAR INSTITUTE

Thirty-sixth Street and Woodland Avenue :: :: Philadelphia, Pa.

MBL/WHOI LIBRARY
WH 17HT H